

Research Note

Effect of Oral Administration of Bismuth Compounds on *Campylobacter* Colonization in Broilers

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ABSTRACT Bismuth compounds have been used since the 18th century to treat gastrointestinal ailments in man. Colloidal bismuth subcitrate (De-Nol) is currently used in combination with antibiotics to reduce enteric *Helicobacter pylori* colonization as a treatment of stomach ulcers. We investigated whether bismuth citrate or its parent compound, colloidal bismuth subcitrate, would reduce colonization of the closely related foodborne pathogen, *Campylobacter jejuni* in chickens. In 2 studies, birds were either fed 0, 50, or 200 ppm bismuth citrate or bismuth subcitrate

(De-Nol) for 10 or 21 d and were orally challenged with 7 combined strains of *C. jejuni* (n = 6 birds/treatment). For both treatment groups, cecal *Campylobacter* colonization was reduced when birds were fed 200 ppm for 10 d but not 21 d. For the 50 ppm treatment group, only birds dosed with bismuth citrate for 21 d demonstrated any reduction in cecal *Campylobacter* concentrations when compared with controls. These data suggest that bismuth citrate and colloidal bismuth subcitrate may reduce cecal colonization by *Campylobacter* in broilers, but these effects are inconsistent.

Key words: chicken, *Campylobacter*, bismuth citrate, bismuth subcitrate, mucin

2006 Poultry Science 85:2009–2011

INTRODUCTION

There are an estimated 2.4 million cases of human *Campylobacter* infections in the United States each year, usually resulting in short-term gastroenteritis but also associated with more chronic diseases such as reactive arthritis and Guillian-Barré syndrome (Friedman et al., 2004). The most common vector of *Campylobacteriosis* in humans is believed to be undercooked poultry, because *Campylobacter* has been frequently isolated from retail poultry (Stern et al., 1988; Fields and Swerdlow, 1999; Mateo et al., 2005). Probiotics have been used with some success to reduce *Campylobacter* contamination in poultry, but the protection has been shown to be inconsistent (Mead, 2002). One strategy that may be worth pursuing is modifying the enteric mucosal environment in which *Campylobacter* normally thrives to reduce the presence of this pathogen. *Campylobacter jejuni* has been shown to be chemotactic to mucin within the crypts of the chicken gastrointestinal tract and utilize mucin as a nutrient source (Beery et al., 1988; Hugdahl et al., 1988). Fernandez et al. (2000) demonstrated that altering mucin characteristics through diet can reduce colonization of *C. jejuni* in broilers. Similarly, bis-

muth compounds have been demonstrated to reduce *Helicobacter pylori*, a bacteria that is closely related to *C. jejuni*, by altering mucin characteristics in humans (Slomiany et al., 1990). It is possible that the modification of chicken mucin with bismuth compounds may also reduce *Campylobacter* colonization in chickens. The medical community has utilized bismuth compounds to treat gastrointestinal ailments for many decades (Scarpignato and Pelosini, 1999). Colloidal bismuth subcitrate (De-Nol, Tri-Med, Subiaco, Australia) is used as a treatment of *H. pylori*-induced peptic ulcers in humans, which has morphological, physiological, and biochemical similarities to *C. jejuni* (Lee, 1991; Newell, 2001). The compound has been demonstrated to increase neutral mucin production, decrease acidic mucin, and inhibit enzymes produced by *H. pylori* to degrade gastric mucin (Lee, 1991). It is also bactericidal for *H. pylori* and has been demonstrated to prevent attachment of *H. pylori* to mammalian epithelial cells (Gorbach, 1990; Lee, 1991; Larsen et al., 2003). Colloidal bismuth subcitrate forms the precipitate bismuth citrate (an active metabolite) when it reaches the acidic environment of the stomach (Wagstaff et al., 1988). The objective of this study was to determine if colloidal bismuth subcitrate or its metabolite, bismuth citrate, affects cecal colonization of *C. jejuni* in young broiler chicks.

MATERIALS AND METHODS

Experimental Birds

Day-of-hatch chicks were obtained from a local commercial hatchery and placed into floor pens with pine shavings

©2006 Poultry Science Association Inc.
Received March 16, 2006.

Accepted June 17, 2006.

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and supplemental heat. Chicks were provided water and a balanced, unmedicated corn-soybean ration ad libitum that met or exceeded the NRC (1994) guidelines for a male broiler diet.

Experimental Design

Study 1. Thirty-six day-of-hatch chicks were separated into 6 separate treatment pens ($n = 6$ birds/treatment) and were fed control feed or feed with bismuth citrate (50 or 200 ppm, Spectrum Chemicals and Laboratory Products, Gardena, CA) for either 10 or 21 d. To ensure *Campylobacter* colonization, all birds were orally challenged with 7 strains of *C. jejuni* 7 d before study termination (3 d for the 10-d treatment group or 14 d for the 21-d treatment group), as described previously by our laboratory (Farnell et al., 2005). The *Campylobacter* challenge was approximately 3.8×10^5 cfu/chick or 1.0×10^7 cfu/chick for birds inoculated at 3 or 14 d of age. At either 10 or 21 d, birds were euthanized, and ceca were aseptically collected for *Campylobacter* quantitation in the following manner: The cecal contents of each bird were serially diluted 1:9 in buffered phosphate diluent, and 100 μ L of each dilution was plated onto *Campylobacter*-Line agar plates (Line, 2001). The plates were incubated for 48 h at 42°C in a microaerophilic environment (5% O₂, 10% CO₂, and 85% N₂). After incubation, characteristic colonies were confirmed as *Campylobacter* using a commercial latex agglutination test kit (Panbio Inc., Columbia, MD) and the *Campylobacter* API biochemical test (Biomérieux, Marcy, l'Etoile, France). The direct counts were converted to log₁₀ colony-forming units per gram of cecal contents. The detection limit for *Campylobacter* was 1×10^2 cfu/g of cecal contents.

Study 2. Study 2 was performed similarly to Study 1 ($n = 6$ birds/treatment), except birds were dosed (0, 50, or 200 ppm) with colloidal bismuth subcitrate (De-Nol, Tri-Med). *Campylobacter* challenge concentrations were 2.9×10^7 cfu/chick or 2.8×10^6 cfu/chick for birds inoculated at 3 or 14 d of age.

Statistical Analysis

Data were analyzed by ANOVA using the Statistical Analysis System (SAS Institute, 2000) general models program. Treatment means were partitioned by least squares means analysis. Colony-forming units of *Campylobacter* were logarithmically transformed before analysis to achieve homogeneity of variance (Byrd et al., 2003; Cole et al., 2004). A probability of $P < 0.05$ was required for statistical significance.

RESULTS AND DISCUSSION

Bismuth compounds have been used to treat gastrointestinal disorders in humans since the 18th century (Scarpignato and Pelosini, 1999). Bismuth drugs used for the treatment of gastrointestinal ailments include Tri-ec, (Glaxo Wellcome, Research Triangle Park, NC), Pepto-Bismol (Proctor & Gamble, Cincinnati, OH), and De-Nol (Tri-Med;

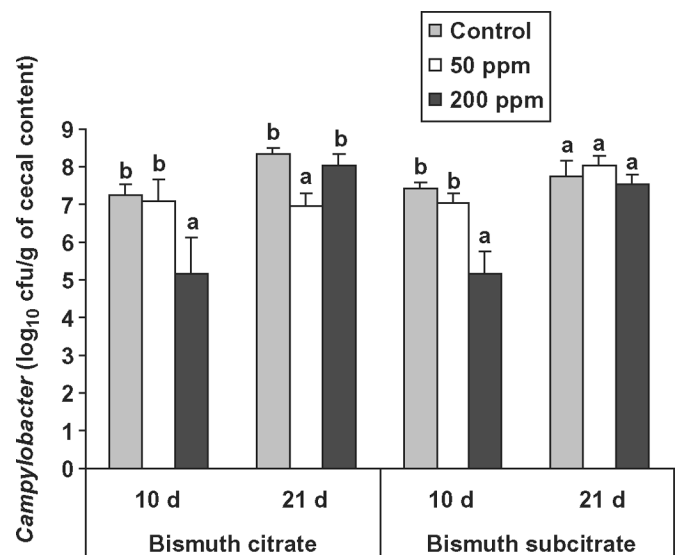


Figure 1. Cecal *Campylobacter* concentration (mean + SEM) in 10- or 21-d broilers treated with either 0, 50, or 200 ppm bismuth citrate or colloidal bismuth subcitrate in the feed. Bismuth citrate or colloidal bismuth subcitrate was fed (0, 50, or 200 ppm) for the entire 10- or 21-d period ($n = 6$ birds/treatment). To ensure *Campylobacter* colonization, birds were challenged with 7 combined strains of *Campylobacter jejuni* 7 d before study termination. *Campylobacter* data are presented as log₁₀ colony-forming units per gram of cecal contents. ^{a,b}Means within treatments and days with no common superscript differ significantly ($P < 0.05$).

Gorbach, 1990; Bland et al., 2004; McLoughlin and O'Morain, 2005). These drugs have antibacterial properties to pathogenic bacteria such as *Escherichia coli*, *H. pylori*, and *Salmonella* (Marshall, 1988; Scarpignato and Pelosini, 1999). Colloidal bismuth subcitrate (De-Nol, Tri-Med) has been used successfully in the treatment of *H. pylori*-induced ulcers in man for over a decade (Rauws et al., 1988; Tillman et al., 1996; Fraser, 2004). In addition to the bactericidal properties of colloidal bismuth subcitrate, it has also been shown to decrease mucin viscosity, prevent bacterial digestion of mucus, and reduce adherence of bacteria to gastric epithelium (Wagstaff et al., 1988). Bismuth citrate is a metabolite of colloidal bismuth subcitrate and was used initially in this study due to its availability and low cost (Wagstaff et al., 1988). Colloidal bismuth subcitrate is not commercially available in the United States and is more expensive than bismuth citrate, which would severely limit its cost:benefit ratio to a commercial poultry operation.

To evaluate the effects of these compounds on *Campylobacter* colonization, chicks were fed 0, 50, or 200 ppm of either bismuth citrate or colloidal bismuth subcitrate for 10 or 21 d. To ensure *Campylobacter* colonization, birds received a *Campylobacter* challenge 7 d before study termination. Feeding 200 ppm of either bismuth citrate or colloidal bismuth subcitrate for 10 d reduced cecal *Campylobacter* concentrations when compared with controls ($P < 0.05$; Figure 1). When birds were dosed with these 2 compounds for 21 d, however, the 200 ppm treatments did not reduce *Campylobacter* concentration. The only effect following the 21-d dosing period was a reduction in *Campylobacter* concentration in the 50 ppm bismuth citrate group (Figure 1).

Results from this study indicate that only short-term treatment (10 d) with 200 ppm of either compound provided a consistent, albeit small, reduction in cecal *Campylobacter* colonization in chickens. Successful use of bismuth compounds against *H. pylori* infections in humans required coadministration of antibiotics such as tetracycline or amoxicillin (Marshall, 1988; Megraud and Marshall, 2000; Bland et al., 2004; Marko et al., 2005). These drug combinations have been optimized to provide a synergistic effect for the treatment of *H. pylori* colonization, and it is possible that a combined treatment may improve the efficacy of colloidal bismuth subcitrate and bismuth citrate for the reduction of *Campylobacter* colonization in broilers. Unfortunately, with the recent concerns about the use of antibiotics and antibiotic resistance (Okeke et al., 2005), this approach may be difficult to implement.

The limited efficacy observed in our study may have been partly due to the relatively high pH of the chicken ceca that has been reported to have a pH of 5.5 to 7.0 (Denbow, 2000). The binding affinity of colloidal bismuth subcitrate to the mammalian gut epithelium increases with reductions in lumen pH (Wagstaff et al., 1988; Lee, 1991). Therefore, colloidal bismuth subcitrate and bismuth citrate may not have bound the cecal epithelium with the affinity necessary to inhibit *Campylobacter* colonization. If a practical means of lowering the pH of the ceca can be found (e.g., organic acids), it may be possible to improve the efficacy of these bismuth compounds.

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